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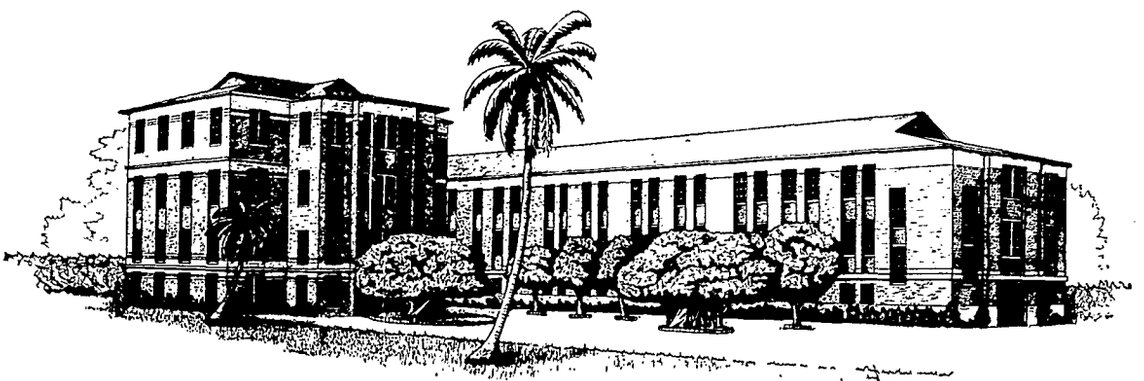

Research Report
MR005.09-1201.12.22

United States Naval
Medical Research Unit No. Two
Taipei, Taiwan

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Classification of Trachoma Virus Strains by Protection of Mice from Toxic Death

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Classification of Trachoma Virus Strains by Protection of Mice from Toxic Death

During the past several years trachoma and inclusion conjunctivitis (TRIC) virus strains have been isolated in many different areas of the world. Attempts to classify and study similarities and differences of these strains are of considerable basic and applied interest. If immunization against trachoma were to become possible, antigenic differences among the strains would be of great importance. Trachoma virus grown to high titer in the yolk sac of the embryonated egg has been shown to be capable of killing mice when injected intravenously. Prevention of toxic death has been achieved by prior inoculation of mice with similar virus strains. With this mouse toxicity prevention test, Bell and his associates[1,2] have demonstrated differences in cross-protection, suggesting at least two groups of trachoma viruses on the basis of this test among those they have isolated in Saudi Arabia and Egypt. By use of this mouse toxicity prevention test (modified), we have studied the relationships of 29 trachoma viruses isolated on Taiwan, three isolated near New Delhi, India, and eight strains (including two considered to be inclusion conjunctivitis) provided by other workers which were isolated on four different continents of the world. Some preliminary results of these studies have been presented.[3]

Materials and Methods

TRIC virus strains: The following 29 trachoma virus strains were isolated from six cities or counties of Taiwan and have been designated as follows:

From Taipei:
Taiwan-2 (abbreviated TW-2 and formerly called TW-21)
TW-4 (formerly TW-89)
TW-5 (formerly TW-97)
TW-7 (formerly TW-215)

From Miaoli:
TW-1 (formerly TW-10)

From Hsinchu:
TW-3 (formerly TW-29)

From Taichung County:
TW-6 (formerly TW-191)
TW-8 (formerly TW-226)
TW-9 (formerly TW-234)
TW-10 (formerly TW-247)
TW-11 (formerly TW-248)
TW-12 (formerly TW-251)
TW-13 (formerly TW-253)
TW-14 (formerly TW-254)
TW-15
TW-16
TW-17
TW-19
TW-29
TW-112
TW-117
TW-128
TW-131
TW-133
TW-134
TW-135
TW-136
TW-137

From Kaohsiung:
TW-54 (formerly TW-339)

Strains TW-1 and TW-2 were isolated in 1958, TW-3 through TW-5 in 1959, TW-6 through TW-54 in 1960, and TW-55 through TW-137 in 1961. Strains TW-131 through TW-137 came from specimens collected in October 1961.

Three strains were isolated at NAMRU-2 from specimens collected in and near New Delhi, India, in March 1960. These are designated ND-1 (formerly ND-112); ND-2 (formerly ND-115) and ND-3 (formerly ND-142).

Four TRIC virus strains received from Drs. P. Thygeson and E. Jawetz are included in these studies. These include the BOUR trachoma strain isolated from a white American in California,[4] two Apache strains (AP-2 and AP-4) isolated from American Indians in Arizona[5] and an inclusion conjunctivitis strain (IC-CAL-3 or Brooks) isolated from a newborn child in San Francisco.[6]

The trachoma strain SA-1 isolated in Saudi Arabia[7] was provided by Dr. J. C. Snyder. Dr. L. H. Collier supplied two trachoma virus strains G-1 and G-17 isolated in Gambia, Africa[8], and one inclusion blennorrhea strain (LB-1) isolated from the cervix of a woman in London.[9]

Virus growth and toxicity titration: All TRIC virus strains were grown in the yolk sac of the embryonated egg. Six to eight-day-old eggs were inoculated with dilutions of stock virus adjusted so that death of embryo occurred eight to ten days later. If a stronger virus inoculum

was used, death of embryo occurred earlier and the yolk sacs were low in toxicity. For the trachoma and IC-CAL-3 viruses, dilution was usually 10^{-2} but LB-1 virus acted quite differently, requiring dilution to 10^{-4} or higher to delay deaths to seven or eight days. When one-third to one-half of the inoculated eggs had died the remaining eggs were harvested, the yolk sacs pooled and a 40% suspension made in sucrose potassium glutamate*.[10] The suspension was distributed in 2-5 ml amounts in screw-capped vials and frozen at -65°C in a mechanical freezer. Yolk sacs from dead eggs were not used for toxicity test because of low potency. A sample of virus pool was thawed and titrated for toxicity in five-week-old (15 Gm) mice. In titrations for minimal lethal toxic dose (TD_{100}), dilution increments of 2.5% or 5.0% of yolk sac were used rather than twofold dilutions. Most pools employed in the tests had a TD_{100} for 15 Gm mice at a dilution of 10%-25% of the original yolk sac. The 50% egg infectious (EID_{50}) and lethal (ELD_{50}) doses were calculated on many of the pools during the earlier part of the study.[11] Material suitable for use in toxicity tests usually had an EID_{50} in the neighborhood of 10^{-6}. The EID_{50} was more closely related to the TD_{100} for mice than was the ELD_{50}.[11] Figure 1 shows titrations of TD_{100} of 43 TRIC virus suspensions on which the EID_{50} was also determined. Suspensions tested represent a number of different virus strains. It should be noted that no purification procedures were carried out on the crude yolk sac virus suspension used for toxicity or protection.

Mice: Mice employed in these studies were an NIH White Swiss strain originally obtained from the Naval Medical Research Institute in Bethesda and maintained in this laboratory.

Protection of mice: The technique evolved for these experiments used intravenous (I. V.) inoculation which was carried out as follows: Three-week-old (8 to 10 Gm) mice of both sexes were used. The first dose was one-third of the TD_{100} in 0.5 ml. The dose was determined from a prior toxicity titration. A second I. V. injection given one week later was calculated to be approximately two-thirds the TD_{100}. With all strains except LB-1, only a few mice (usually 10-15%) were killed during the protection procedure. With the LB-1 strain, large numbers of

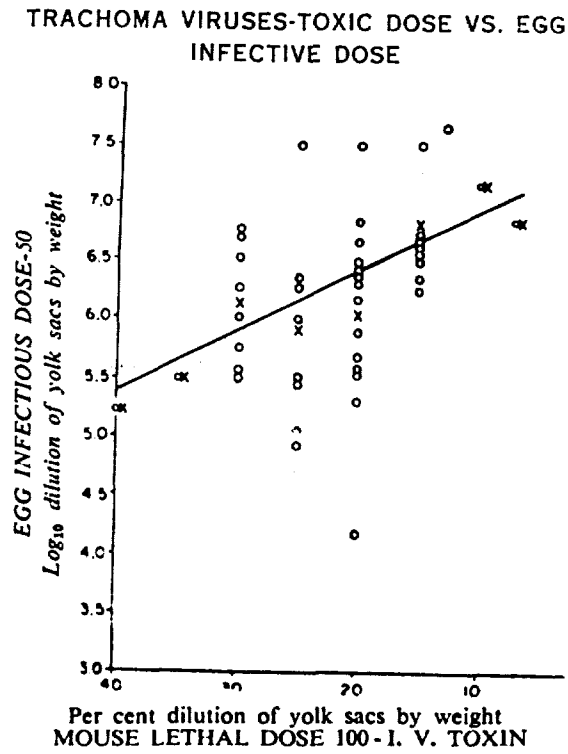

Figure 1. This graph shows the relationship between the minimal lethal mouse toxic dose and the 50% egg infectious dose of 43 TRIC virus crude yolk sac suspensions.

mice (up to 50%) died. After an additional seven to ten days the mice were challenged.

Toxicity test: The TD_{100} dose previously determined for the pool was used for challenge. Mice were injected I. V. with 0.5 ml. Deaths occurring after one hour and up to 24 hours (occasionally up to 48 hours) were due to toxicity; on gross examination the peritoneal and intestinal vessels were seen to be engorged and the liver and intestines were dark brown. Previously uninoculated mice of the same age (about five weeks old) as those who had received the two sublethal injections were included in each test as controls and unless at least three-fifths of the control mice were killed by the challenge inoculation, results of the experiment were discarded. In cross- protection tests if at least 40% protection was not observed in mice given homologous strain injections, the results in that experiment with other mice previously inoculated with that strain were discarded.

* Sucrose 75 Gm; KH_2PO_4 0.52 Gm; Na_2HPO_4 1.22 Gm; glutamic acid 0.72 Gm; distilled water to make 1000 ml adjusted to pH 7.4 to 7.6 and autoclaved 15 lbs 15 minutes.

Technical considerations: In early experiments with the mouse toxicity prevention test, considerable difficulties were encountered in obtaining clear-cut results. Protection was often inadequate and challenge doses were too strong or too weak. In addition, an irregular but often large number of anaphylactic deaths occurred within one hour after challenge. We were unable to employ any purification methods and still maintain material of adequate toxicity. With the development of virus pools of greater toxicity that could be frozen and titrated before use, many technical difficulties disappeared. Experiments were carried out with intraperitoneal, intracerebral and I. V. routes for protective injection and it was found that I. V. inoculation gave better protection and more reproducible results. Anaphylactic deaths have occurred only rarely since frozen virus suspensions have been used via the I. V. route. Careful prior titration is essential because too strong a challenge dose will overwhelm protection either from a homologous strain or a heterologous cross-protecting strain. Even in well-controlled experiments some irregularity of results with the mouse toxicity prevention test may occur and it is recommended that results of a single test classifying a new strain be confirmed at least once in a separate test before the classification is accepted. Because of the use of very high titer live virus, the use of goggles is recommended during intravenous inoculations in mice.

Results

An example of toxicity titration of a crude yolk sac pool of Gambia trachoma strain G-17 (Pool #6) and two successive mouse toxicity protection tests using mice previously injected with this strain are shown in Table 1. The minimum TD_{100} of the virus pool was estimated to be 10% on the basis of the titration (A). For protection mice were inoculated with 3.5% and a week later with 7% of this crude yolk sac pool. Seven days later the inoculated mice were challenged with ten TRIC virus strains including the homologous strain using the previously determined TD_{100}. Five uninoculated controls of the same age were challenged with each strain along with eight protected mice (B). All control mice were killed with each strain except one challenged with IC-CAL-3. Six of eight mice were protected from death against the homologous G-17 and the SA-1 strains. Three of eight mice challenged with G-1 lived. There was no protection against the other challenge strains. Another cross-protection test was performed with mice protected with pool #6 of strain G-17. The four challenge strains included the homologous G-17, a repeat of G-1, and two Apache strains. Again, all control mice were killed. Nine of ten mice were protected against the homologous strain, but there was no protection with the other strains. This set of experiments demonstrated that prior sublethal injections with G-17 protected against challenge with G-17 and SA-1 strains but not against ten other TRIC virus strains tested.

A series of similar experiments were carried out with 19 Taiwan trachoma virus strains and a compilation of these experiments is presented in Table 2. The actual number of mice challenged and the number protected from toxic death are shown. On the basis of comparison of the pattern of protection, two groups emerge among these 19 Taiwan strains. The strains within each group cross-protect again challenge by members of that group. The TW-1 virus has been designated the prototype strain for one group and the TW-3 strain for the other group. Because of the small number of mice involved in some of the cross challenge tests, it is difficult to make percentage comparison; however, when results are grouped, the extent of differences and similarities among the strains can be more clearly expressed. In Table 3 a summary of the data of Table 2 is presented with 16 of the viruses shown both as challenge strain and as protecting strain against members of the TW-1 and TW-3 groups. Sharp separation of the two groups is demonstrated, with the important comparison being the difference in percentage protection between the homologous and the heterologous groups. Strains TW-15, TW-16 and TW-19 were tested only as challenge strains. On the basis of this one-way test, TW-15 and TW-16 fell clearly into the TW-3 group and TW-19 into the TW-1 group.

A second series of experiments with ten more recently isolated Taiwan strains has been completed. The results with these ten strains are shown in Table 4. The prototype Taiwan strains, TW-1 and TW-3, along with one other strain from each group (TW-5 and TW-54), were used for comparison in these cross-protection tests. A percentage protection summary of the data shown in Table 4 is presented in Table 5. Again, it was demonstrated that all of the Taiwan strains fall clearly into one or the other of the two groups.

Table 1. Example of a toxicity titration and cross-protection tests with Gambia trachoma strain G-17 and 11 other TRIC viruses.

A. MOUSE TOXICITY TITRATION OF G-17 POOL #6: 11-25-61

Yolk Sac Virus Concentration	Death in Hours					D/T	% Mortality
20%	2	18	18	18	18	5/5	100
15%	3	18	18	18	18	5/5	100
10%	18	18	18	18	18	5/5	100
5%	18	—	—	—	—	1/5	20

B. PROTECTION INJECTIONS (3.5%: 12-8-61, 7.0%: 12-15-61 & CHALLENGE (12-22-61)

Challenge	Strain	Mice Protected with C-17 Death in Hours								D/T	Control Mice Death in Hours					D/T	% Protection
TW-1(P# 23)	20%	5	6	7	8	18	18	18	18	8/8	7	18	18	18	18	5/5	0
TW-5(P# 4)	5%	5	7	18	18	18	18	18	—	7/8	7	18	18	18	18	5/5	13
TW-3(P# 27)	20%	5	5	5	6	7	18	18	18	8/8	5	5	8	8	18	5/5	0
ND-3(P# 4)	15%	(<1)	18	18	18	18	18	18	48	7/7	5	18	18	18	18	5/5	0
BOUR(P# 16)	15%	5	7	18	18	18	18	18	18	8/8	5	7	7	7	8	5/5	0
SA-1(P# 15)	15%	2	5	—	—	—	—	—	—	2/8	4	4	4	4	4	5/5	75
G-17(P# 6) (homologous)	10%	18	18	—	—	—	—	—	—	2/8	5	7	18	18	18	5/5	75
G-1(P# 7)	15%	18	18	18	18	48	—	—	—	5/8	7	18	18	18	18	5/5	33
IC-CAL-3(P# 2)	10%	18	18	18	18	18	18	18	—	7/8	6	6	6	7	—	4/5	13
LB_1 (P# 4)	10%	18	18	18	18	18	18	18	—	7/8	18	18	18	18	18	5/5	13

C. PROTECTION INJECTIONS (3.5%: 1-3-62, 7.0%: 1-10-62) & CHALLENGE (1-18-62)

Challenge	Strain	Protected with G-17 Death in Hours					D/T	Control Mice Death in Hours					D/T	% Protection
G-17(P# 6) (homologous)	10%	18	—	—	—	—	1/10	4	6	6	7	7	5/5	90
		—	—	—	—	—								
G-1(P# 7)	15%	6	7	18	18	18	10/10	6	7	7	7	18	5/5	0
		18	18	18	18	18								
AP-2(P# 2)	18%	4	4	5	6	7	10/10	3	3	5	5	18	5/5	0
		7	7	18	18	18								
AP-4(P# 1)	40%	5	6	18	18	18	9/10	7	18	18	18		4/4	10
		18	18	18	29	—								

Note: Immunization and protection injection were 0.5 ml of virus dilution by intravenous route.
— = mouse alive at 48 hours
D/T = number of deaths per total number challenged

The low homologous protection with the TW-3 strain in two experiments in this series of tests is an example of the variability that may be encountered even with well-titered challenge virus. The protection was probably caused by too strong a challenge since other mice immunized with TW-3 in the same experiment were protected against TW-3 group strains.

Among the Taiwan strains tested, there were two pairs of strains from the same person. TW-11 and TW-13 were isolated from the same seven-year-old boy one month apart. TW-16 and TW-131 were isolated 16 months apart from a boy who was six years old at the time of the first isolation.

Table 2. Cross-protection experiments with 19 Taiwan trachoma virus strains is the mouse toxicity prevention test.

PROTECTING STRAIN	CHALLENGE STRAIN: TW-1 Group							TOTAL TW-1 GROUP	TW-3 Group											TOTAL TW-3 GROUP
	TW-1	TW-5	TW-7	TW-8	TW-10	TW-12	TW-19		TW-2	TW-3	TW-4	TW-6	TW-9	TW-11	TW-13	TW-14	TW-15	TW-16	TW-17	TW-54	
TW-1	**102/110***	15/16	15/22	11/15	7/16	9/16	9/10	66/95	0/7	1/44	2/12	1/10			2/6	5/10	3/18	0/4	2/14	0/10	16/135
TW-5	30/35	**56/62**		7/9	4/9	15/25		56/78	1/6	1/25			0/17						1/8	0/10	3/66
TW-7	8/8	3/5	**18/20**	2/4	4/7			17/24		1/7	3/10	0/5				1/4					5/26
TW-8	15/16	11/14	3/5	**28/31**	6/10	9/9	4/4	48/58		0/6				0/6	0/4	9/20			0/7		9/43
TW-10	8/10	4/7		6/6	**17/22**	13/16		31/39		0/10			3/14	0/4			1/4		0/6	4/14	8/52
TW-12	11/15	5/8		9/9	7/9	**15/15**		32/41		1/6		0/4		0/4	0/4		1/6		3/9		5/33
TOTAL TW-1 Group	72/84	38/50	18/27	35/43	28/51	46/66	13/14		1/13	4/98	5/22	1/19	3/31	0/14	2/14	15/34	5/28	0/4	6/44	4/34	
TW-2	0/4	0/5		0/5	1/3			1/17	**12/16**	12/15	15/18	8/12									35/45
TW-3	9/44	0/23	0/5		0/5	0/3		9/80	17/18	**106/132**	23/27	5/7				6/7		2/2	8/11	9/9	70/91
TW-4	0/3	0/5	1/6	0/4	0/4		1/6	2/28	4/4	11/19	**24/27**						3/4	2/4			20/31
TW-6	1/8				0/4			1/12	4/7	6/10	3/3	**14/21**		4/4							17/24
TW-9	0/7		10/10		1/8	1/7	0/11	2/43		6/6	8/12	8/8	**8/8**					10/10		8/8	40/44
TW-11	3/11				2/10	2/10		6/31		6/6			8/8	**14/18**	3/4	5/6	2/4	3/5		8/8	35/41
TW-13	0/6				0/4	2/11		2/21		3/6	2/3	3/4		4/4	**8/12**		3/4				15/21
TW-14	2/12		1/10	2/6		0/5		5/33		5/6			6/6		5/5	**8/10**	4/5				20/22
TW-17	0/8	0/8		0/7	0/7	0/7		0/37		18/24									**21/26**	23/24	41/48
TW-54	1/10				4/28			5/38		17/20			7/7	25/27					4/8	**23/27**	53/62
TOTAL TW-3 Group	16/113	0/41	2/31	2/22	8/73	4/43	1/17		25/39	84/112	51/63	24/31	21/21	33/35	8/9	11/13	12/17	17/21	12/19	48/49	

* Number of mice surviving toxic challenge over number challenged.

Table 3. Percentage protection summary of mouse toxicity prevention tests with 19 Taiwan trachoma virus strains.

TRACHOMA VIRUS STRAIN	Homologous Strain		Per cent of Mice Protected from Death: PROTECTING STRAINS TW-1 Group	PROTECTING STRAINS TW-3 Group		CHALLENGE STRAINS TW-1 Group	CHALLENGE STRAINS TW-3 Group
TW-1	93		86	14		69	12
TW-5	90		76	0		72	5
TW-7	90		67	6		71	19
TW-8	90		81	9		83	21
TW-10	77		55	11		79	15
TW-12	100	CHALLENGE STRAIN	70	9	PROTECTING STRAIN	78	15
TW-19	—		93	6		—	—
TW-3	80		4	75		6	78
TW-2	75		8	64		11	77
TW-4	89		23	81		7	65
TW-6	67		5	77		8	71
TW-9	100		10	100		5	91
TW-11	78		0	94		19	85
TW-13	67		14	89		10	71
TW-14	80		44	85		15	91
TW-15	—		18	71		—	—
TW-16	—		0	81		—	—
TW-17	81		14	63		0	85
TW-54	85		12	98		13	85

Table 4. Cross-protection experiments with an additional 10 Taiwan trachoma virus strains in the mouse toxicity prevention test.

PPOTECTING STRAINS	CHALLENGE STRAINS TW 1	TW 5	TW 112	TW 128	TW 134	TW 135	TOTAL	TW 3	TW 54	TW 117	TW 131	TW 29	TW 133	TW 136	TW 137	TOTAL
TW-1	**15/20**		7/20	5/8	11/20	11/19	34/67		0/10	0/8	0/9	1/9	0/10	1/9	1/10	3/65
TW-5		**13/19**	13/17	7/9	10/17	12/17	42/60	0/10		0/9	0/9	0/9	0/9	1/9	0/9	1/64
TW-112	10/10	6/8	**8/10**	6/8	9/10	8/10	39/46	2/6	5/10	1/10	1/9	1/8	2/10	1/10	1/7	14/73
TW-135	9/9	7/9	9/9	9/9	7/9	**9/10**	41/45	4/8	6/9	5/8	5/9	3/8	4/9	1/8	2/9	30/68
TOTAL	19/19	13/17	29/46	27/34	37/56	31/46		6/27	11/29	6/35	6/36	5/34	6/38	4/36	4/35	
TW-3		0/6	0/8	0/8	4/16	0/8	4/46	**6/18**		10/17	6/16	7/8	7/8	7/8	4/5	41/62
TW-54	0/8		0/7	0/8	0/7	0/6	0/36		**16/18**	9/18	13/18	6/8	5/7	5/8	6/7	44/66
TW-117	2/17	0/17	1/17	0/17	0/17	1/17	4/102	7/16	15/17	**13/16**	11/17	8/17	9/17	9/17	11/17	70/118
TW-137	2/10	0/10	1/10	0/10	0/10	0/10	3/60	8/10	10/10	6/10	6/10	8/10	8/10	7/10	**10/10**	53/70
TOTAL	4/35	0/33	2/42	0/43	4/50	1/41		15/26	25/27	25/45	36/61	29/43	29/42	28/43	21/29	

* Number of mice surviving toxic challenge over number challenged.

Tracnoma strains isolated from other parts of the world have been similarly tested in the mouse toxicity prevention test. Five strains that cross-protect with Taiwan strains have been found. Table 6 is a percentage protection summary of the data with these five strains and in addition shows the number of mice challenged. The three trachoma virus strains from New Delhi were found to protect against one another and to cross-protect with strains of the TW-3 group. There was no cross-protection with the TW-1 group or with any of six other TRIC viruses not

Table 5. Percentage protection summary of mouse toxicity prevention tests with 10 additional Taiwan trachoma virus strains.

TRACHOMA VIRUS STRAIN	Per cent of Mice Protected from Death						
	Homologous Strain	CHALLENGE STRAIN	PROTECTING STRAINS		PROTECTING STRAIN	CHALLENGE STRAINS	
			TW-1 Group	TW-3 Group		TW-1 Group	TW-3 Group
TW-1	75		100	11		51	5
TW-5	68		76	0		70	2
TW-112	80		63	5		85	19
TW-128	—		79	0		—	—
TW-134	—		66	8		—	—
TW-135	90		67	2		91	44
TW-3	33		22	58		9	66
TW-54	89		38	93		0	67
TW-117	81		17	55		4	59
TW-131	—		17	59		—	—
TW-29	—		15	67		—	—
TW-133	—		16	69		—	—
TW-136	—		11	65		—	—
TW-137	100		11	72		5	76

Table 6. Summary of mouse toxicity prevention tests with three New Delhi and two American Indian trachoma virus strains cross tested with prototype Taiwan trachoma strains and six TRIC viruses not belonging to the Taiwan groups.

New Delhi & Arizona Trachoma Strains	Per cent of Mice Protected from Death							
	Homologous Strain	CHALLENGE STRAIN	PROTECTING STRAINS			PROTECTING STRAIN	CHALLENGE STRAINS		
			TW-1 Group	TW-3 Group	Other Strains		TW-1 Group	TW-3 Group	Other Strains
ND-1	67 (6)*		11 (18)	70 (30)	0 (28)		7 (14)	70 (10)	12 (26)
ND-2	67 (6)		15 (26)	79 (14)	3 (34)		0 (3)	53 (17)	8 (26)
ND-3	84 (25)		13 (31)	74 (34)	7 (84)		18 (34)	81 (31)	7 (103)
AP-2	68 (28)		65 (55)	0 (19)	20 (81)		83 (84)	7 (15)	5 (50)
AP-4	55 (20)		72 (60)	0 (9)	14 (37)		67 (60)	10 (20)	11 (70)

* ( ) shows number of mice challenged.

belonging to the two Taiwan groups. Another recently isolated (1962) New Delhi strain (ND-4) has been shown to cross-protect with the TW-3 group. The two Apache strains were found to protect against each other and TW-1 group strains. There was no cross-protection with the TW-3 group or with the six other TRIC viruses.

Using AP-2 and ND-3 as representative strains along with three Taiwan strains, a series of cross-protection tests were carried out with four trachoma strains and two inclusion con-

Table 7. Cross-protection experiments in the mouse toxicity prevention test with 11 TRIC virus strains from 6 different countries.

PROTECTING STRAIN	CHALLENGE STRAIN										
	TW-1	TW-5	AP-2	TW-3	ND-3	SA-1	G-17	G-1	BOUR	LB-1	IC-CAL-3
TW-1	**96/111***	23/26	13/20	0/20	3/15	2/22	4/25	7/33	10/67	3/43	2/9
TW-5	18/23	**31/34**	10/15	1/14	1/16	3/19	0/9	3/20	0/10	0/18	0/10
AP-2	24/29	21/28	**19/28**	0/8	1/7	0/6	0/9	0/9	0/9	0/5	3/18
TW-3	0/9	0/19	0/9	**48/57**	23/30	2/22	4/13	2/14	3/25	0/9	0/9
ND-3	6/25	0/9	0/10	18/21	**21/25**	0/12	2/19	3/23	0/10	0/9	2/20
SA-1	2/14	0/7	0/8	0/10	7/26	**46/55**	25/30	5/16	1/15	0/10	1/9
G-17	0/13	1/8	0/10	1/20	1/11	21/30	**47/62**	8/55	0/13	1/8	1/8
G-1	2/10	1/8	0/8	0/15	0/14	0/37	1/38	**39/49**	1/15	2/10	0/9
BOUR	10/50	0/10	0/10	2/26	1/16	5/25	1/22	6/29	**55/75**	8/48	1/10
LB-1	16/35	7/25	11/25	1/13	1/15	0/13	1/14	2/23	2/26	**45/60**	1/10
IC-CAL-3	5/25	0/19	5/20	0/19	0/10	5/20	0/10	2/20	0/10	2/16	**26/30**

* Number of mice surviving toxic challenge over number challenged.

Table 8. Percentage protection summary of mouse toxicity prevention tests with 11 TRIC viruses from around the world.

TRIC virus strains	Per cent of Mice Protected from Death										
	Homologous Strain		PROTECTING STRAINS					CHALLENGE STRAINS			
			TW-1 Group	TW-3 Group	SA-1 Group	Other Strains		TW-1 Group	TW-3 Group	SA-1 Group	Other Strains
TW-1	86	CHALLENGE STRAIN	81	18	7	27	PROTECTING STRAIN	78	9	13	14
TW-5	91		82	0	7	13		74	7	11	5
AP-2	68		66	0	0	25		79	7	0	7
TW-3	84		2	86	3	4		0	77	17	9
ND-3	84		13	77	22	4		14	86	6	8
SA-1	84		11	6	70	11		7	19	83	14
G-17	76		9	19	83	4		3	6	70	12
G-1	80		16	14	18	14		12	0	1	9
BOUR	73		12	9	4	6		14	7	13	17
LB-1	75		5	0	6	16		40	7	4	8
IC-CAL-3	87		14	7	12	7		16	0	17	9

junctivitis strains from other areas of the world. Results of these experiments are shown in Table 7. These tests demonstrate that the strains SA-1, G-17, G-1, BOUR, LB-1 and IC-CAL-3 do not belong to the two cross-protecting groups of which TW-1 and TW-3 are prototype strains. It was found that the SA-1 and G-17 strains cross-protect, thus establishing a third group with at least two trachoma viruses. Four strains (G-1, BOUR, LB-1, IC-CAL-3) failed to cross-protect with any of the other viruses tested. A percentage protection summary of the data in Table 7 is presented in Table 8 where the cross tests are compiled by the three groups plus the four ungrouped strains. Complete delineation of the groups is demonstrated with one exception; this was with the LB-1 strain. When TW-1 virus strains were used for protection, there was no protection against toxic challenge with the LB-1 virus. However, when the LB-1 strain was used as the protecting strain and challenged with TW-1 group viruses, partial protection (40%) occurred. The significance of this partial, one-way cross-protection is unknown.

Discussion

It would appear likely that only two groups of trachoma viruses, as demonstrated in the mouse toxicity prevention test, exist on Taiwan. The 29 Taiwan trachoma strains tested were isolated over a four year period and from six different geographical areas on Taiwan. With the test employed, no differences could be demonstrated between those viruses isolated in 1958 and 1959 and those obtained in late 1961. It was of interest that about 40% of the strains belong to the TW-1 group both from among those tested in the early group of isolates and those of the later group. There were no differences among the persons from whom TW-1 and TW-3 group strains were isolated as far as sex, age, place of residence or severity of clinical symptoms. The finding of only two cross-protection groups of trachoma strains on Taiwan is encouraging because the two prototype strains of these groups were employed in a bivalent experimental trachoma virus vaccine field tested on Taiwan.[12]

The finding that four trachoma strains from areas of the world farthest from Taiwan failed to cross-protect with the two Taiwan groups or with each other except for one pair (G-17 and SA-1), suggests that the number of different groups of trachoma viruses in this test is at least five and may be more. Since a large number of strains has been shown to fall into the two Taiwan groups, it may be concluded hopefully that the strain differences do have geographic patterns and that the total number of different cross-protecting groups may not be great. The fact that two inclusion conjunctivitis strains failed to cross-protect with any of the trachoma strains would suggest that the biological differences attributed to inclusion conjunctivitis virus may have further expression in different mouse toxins. It is also of interest that the BOUR strain which has been shown to be unique in its high pathogenicity for the monkey eye[13, 14] does not cross-protect with any strains tested.

Bell and his colleagues[1, 2] have classified 16 trachoma virus strains into two groups. They studied three strains (SA-1, BOUR, TW-1) that were also included in these experiments. They classified the BOUR and TW-1 strains in the same group. Whether this conflict in findings is due to technical irregularities common with the test or to differences in methods employed by the two laboratories is unknown. There are several important differences in the tests used by Bell and associates and by us. They used partially purified virus preparations both for vaccination and challenge and their toxic challenge dose averaged 200% of original yolk sac material. Their vaccine was formalinized and given intraperitoneally in three doses, a total of 21 days apart. The percentage of protection found even from the series of three injections was generally much lower than the protection reported in this paper. It is not clear whether the protection afforded by prior sublethal I. V. injection is mediated through an immune mechanism with the production of antibodies or antitoxin, or whether the receptor sites for toxic effect are rendered less susceptible through some other mechanism. The test as described in this report shows clear specificity among TRIC viruses and if it differs from Bell's test, it is in the direction of greater specificity.

The question of whether differences in TRIC virus strains demonstrated in the mouse toxicity prevention test are significant in protection with vaccine against infection remains to be demonstrated. Preliminary results in this laboratory from successful vaccine protection studies in monkeys against eye infection with trachoma viruses would suggest that these strain differences are significant.

Summary

Twenty-nine trachoma virus strains isolated on Taiwan and nine trachoma virus strains and two inclusion conjunctivitis virus strains obtained from other areas of the world have been tested in mice for cross-protection against intravenous toxicity. Crude yolk sac suspensions of these TRIC viruses were used both for protection and challenge. Protection was produced by intravenous injection of one-third and then two-thirds a minimum lethal toxic dose 14 and 7 days prior to challenge. All 29 of the Taiwan trachoma strains fell into one of two cross-protecting groups designated as the TW-1 and TW-3 groups. Three trachoma strains from New Delhi protected against each other as well as members of the TW-3 group. Two trachoma strains from Apache Indians in Arizona cross-protected with the TW-1 group. Four trachoma and two inclusion conjunctivitis strains failed to cross-protect with the Taiwan groups. The SA-1 and G-17 strains protected against each other but not against the other strains tested. The G-1 and BOUR trachoma viruses and the LB-1 and IC-CAL-3 inclusion conjunctivitis viruses showed no cross-protection with any of the strains. It is concluded that the total number of different groups of TRIC virus strains that can be demonstrated with the mouse toxicity prevention test are not yet known but that in a given geographical area as exemplified by Taiwan, the number of different antigenic groups is probably limited.

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